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Adenocarcinoma of the pancreas: Comparative single centre analysis between ductal and mucinous type.

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1. Background

Adenocarcinomas of the pancreas are exocrine tumors, originate from ductal system, including two morphologically distinct entities: the ductal adenocarcinoma and mucinous adenocarcinoma. Ductal adenocarcinoma is by far the most frequent malignant tumor in the pancreas, representing at least about 90% of all pancreas cancers. It is associated with very poor prognosis, due to the fact that actually there are no any biological markers or diagnostic tools for identification of the disease at an early stage. Most of the time the disease is extensive with vascular and nerves involvement or with metastatic spread at the time of diagnosis (1). The median survival is less than 5% at 5 years, placing it, at the fifth leading cause of death by cancer in the world (2). The mucinous form of pancreatic adenocarcinoma is less frequent, and seems to have a better prognosis with about 57% survival at 5 years (1)(3)(4).

Each morphologic type of pancreatic adenocarcinoma is associated with particular preneoplastic lesions. Two types of preneoplastic lesions are described: firstly, pancreatic intra-epithelial neoplasia (**PanIN**) which affects the small and peripheral pancreatic ducts, and the intraductal papillary-mucinous neoplasm (**IPMN**) interested the main pancreatic ducts and its principal branches. Both of preneoplastic lesions lead by different mechanisms to the pancreatic adenocarcinoma (1)(2)(3)(4)(5)(6)(7)(8)(9)(10).

The purpose of our study consists in a retrospective analysis of various clinical and histo-morphological parameters in order to assess a difference in survival between these two morphological types of pancreatic adenocarcinomas.

1.2 Material and methods

We conducted a retrospective analysis including 35 patients, (20 men and 15 women), beneficed the surgical treatment for pancreas adenocarcinoma at the Surgical Department of University Hospital in Lausanne. The patients involved in our study have been treated between 2003 and 2008, permitting at least 5-years mean follow up. For each patient the following parameters were analysed: age, gender, type of operation, type of preneoplastic lesions, TNM stage, histological grade of the tumor, vascular invasion, lymphatic and perineural invasion, resection margins, and adjuvant treatment.

The results from these observations were included in a univariate and multivariate statistical analysis and compared with overall survival, as well as specific survival for each morphologic

subtype of adenocarcinoma.
As a low number of mucinous adenocarcinomas (n=5) was insufficient to conduct a pertinent statistical analysis, we compared the data obtained from adenocarcinomas developed on PanIN with adenocarcinomas developed on IPMN including both, ductal or mucinous types.

1.3 Result

Our results show that adenocarcinomas developed on pre-existing IPMN including both morphologic types (ductal and mucinous form) are associated with a better survival and prognosis than adenocarciomas developed on PanIN.

1.4 Conclusion

This study reflects that the most relevant parameter in survival in pancreatic adenocarcinoma seems to be the type of preneoplastic lesion. The significant difference in survival was noted between adenocarcinomas developing on PanIN as compared to adenocarcinomas developed on IPMN precursor lesions. Ductal adenocarcinomas developped on IPMN present significantly longer survival than those developed on PanIN lesions (P value= 0,01). Therefore we can suggest that the histological type of preneoplastic lesion rather than the histological type of adenocarcinoma should be the determinant prognosis factor in survival of pancreatic adenocarcinoma.

2. Introduction

Pancreatic adenocarcinoma is currently the five leading cause of death by cancer in the world (2). This is explained in part by the silent development of the disease. The symptoms such as ictere or weight loss appear in advanced stages of disease (1). The pancreas is, due to its anatomic localisation, more difficultly accessible to screening techniques as compared for example to other organs such breast and colon. The new screening techniques such Endoscopic Retrograde Cholangio-Pancreatography (ERCP) allows evaluation of the biliary and pancreatic ductular system. However, it is an invasive technique leading to the risk of pancreatitis in more than 10% of cases (13). Most of the time the disease is discovered at a

relatively advanced stage, which explains the poor prognosis with the median survival being less than 5% at 5 years (1)(5).

The various risk factors such age, smoking, ethylism, chronic pancreatitis, obesity and familiar history are known to predisposing pancreatic adenocarcinoma(1)(5).

The pancreatic adenocarcinoma is usually associated with preneoplastic lesions. The classification of these lesions was for a long time complex, since there was no uniform consensus concerning their origins and their significance in the outcome of patients. In 2003 an official classification of pancreatic intraepithelial neoplasia (**PanIN**) and intracanalar papillary mucinous neoplasia (**IPMN**) was adopted (8).

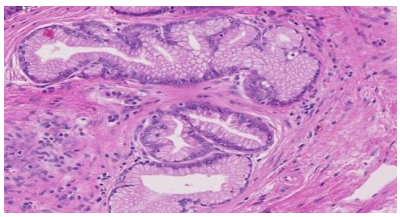
PanINs are peripheric lesions, affecting the small pancreatic ducts, less than 0,5 cm in diameter, involved always in conventional, ductal type adenocarcinoma. According to degree of dysplasia the PanIN lesions are grouped into 3 categories.**(Table 1)**

IPMN lesions are less frequent than PanIN, and occur most often in the main pancreatic duct and its principal branches (3). As these lesions are larger than 0,5 cm in diameter, they can be detected easily by Rx. The IPMNs are also characterized by significant production of mucin. Morphologically, IPMN present the papillary architecture (rarely flat), consisting of columnar epithelial cells with different degrees of dysplasia (8). As PanIN, IPMN appear to follow an adenoma-carcinoma sequence according to the degree of dysplasia as shown in **Table 1**.

There are three categories of IPMNs: low grade adenoma, borderline neoplasia, and carcinoma in situ (7). The prognosis of these lesions seem to be less severe than for PanIN. IPMN lesions lead to cystic formation with mucin production, and affected the large size ducts, permitting to be detected by conventional radiological techniques at a earlier stage (1)(8). They are classified in four histological subgroups, and in the most of cases, they evolve to the mucinous adenocarcinoma (1)(3)**)**(7).

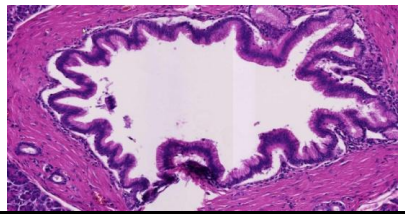
The most common form is the *intestinal type* of IPMN. It represents a villous appearance similar to villous adenoma of the colon. It grows most of the time in the main duct of the pancreas. In case of progression, it will develop into a mucinous adenocarcinoma. The *pancreatobiliary type* is a rare form showing papillary structures and evolves toward the ductal adenocarcinoma. This type has a less well prognosis as compared to intestinal type. The *oncocytic type* seems to be similar to the pancreatobilary type with the presence of oncocytic epithelial features and papillary structures. The evolution of this lesion remains still unknown. The *gastric type* is lined by cylindrical epithelial cells look like gastric foveolar cells with glandular elements resembling to pyloric glands at the top of the papillae. It seems that this type of IPMN lesion is the less aggressive (7).

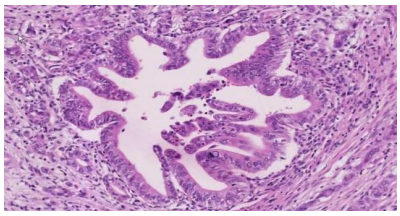
2.2 Table 1: Nomenclature of preneoplastic lesions



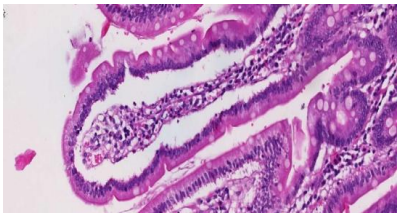
PanIN-1A:
(**Pan**creatic **I**ntraepithelial **N**eoplasia **1-A**): flat lesion, tall columnar cells, basally located nuclei, abundant supranuclear mucin, small nuclei round to oval in shape.
PanIN-1B: papillary, micropapillary or basally pseudostratified architecture, but are otherwise identical to PanIN-1A.

PanIN-2: mucinous epithelial lesions flat or papillary, have some nuclear abnormalities such as loss of polarity, nuclear crowding, enlarged nuclei, pseudo-stratification and hyperchromatism. Mitoses are rare, but when present not apical and not atypical.

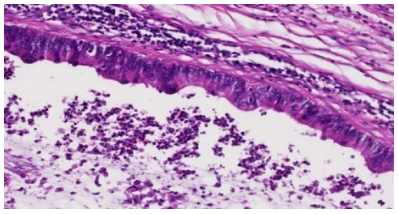




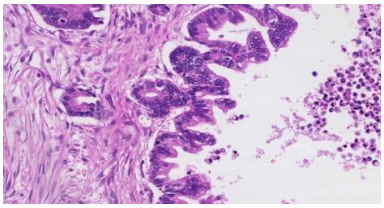
PanIN-3: papillary or micropapillary, cribriforming, budding loss of nuclear polarity, dystrophic goblet cells abnormal mitoses nuclear irregularities and prominent nucleoli.



IPMN adenoma: tall columnar epithelium with mucin-containing cells slight or no atypia.



IPMN borderline: moderate dysplasia, papillary areas and pseudopapillary structures.



Intraductal papillary mucinous carcinoma: severe dysplasia (carcinoma in situ) absence of invasion, with papillary or miocropapillary patter, cribiform growth and budding of small clusters of epithelial cells lack of mucin.

Usually it is not difficult to distinguish the PanIN from the IPMN lesions, because of their different sizes and localization. However, the distinction can be sometimes difficult, as the gastric type IPMN, for example, may extend to small ducts, and inversely some PanIN lesions can extend into the main and branches pancreatic ducts (8).
It is not uncommon for the IPMN lesions to develop into a ductal adenocarcinoma (8). However it may be possible to distinguish the initial preneoplastic lesion by its particular immunophenotype. Both types of lesions (PanIN and IPMN) produce mucin, which is a glycoprotein of high molecular weight. Mucin profile is different in these two lesions and may be of particular interest to distinguish various histological types of ductal pancreatic neoplasia.
MUC1 is essentially expressed in PanIN lesions, and its expression increases with the severity of dysplastic changes leading to ductal adenocarcinoma. Expression of MUC1 is associated with poor prognosis. MUC2 is more characteristic for IPMN lesions. However the gastric type of IPMN express MUC1 and not MUC2 and in this case the difference between PanIN and IPMN is more difficult (1)(2)(6)(7)(9)(10)(11).

Both preneoplastic lesions, PanIN and IPMN, follow a sequence of events leading to adenocarcinoma. The tumor progression will be different depending on the preneoplastic lesion. Ductal adenocarcinoma more often arising in PanIN, while a mucinous adenocarcinoma developped more often on IPMN (1)(3)(12). However, the prognosis seems to be different between these two histological types of adenocarcinomas. While for the ductal adenocarcinoma survival at 5 years is less than 5% (5)(1), it leads about 57% for mucinous adenocarcinoma (4).
In this study we analyze different parameters and tumor behaviour that could explain the difference in survival betweenthese two groups of pancreatic adenocarcinoma.

3. Material and methods

35 patients, (20 men and 15 women) treated by surgery for pancreatic adenocarcinoma in the department of visceral surgery at University Hospital of Lausanne (CHUV), between 2003 and 2008 were selected for this study. A retrospective analysis were carried out using several parameters: age, gender, type of operation, histological type of tumor, preneoplastic lesions, TNM stage, tumor grade, lymphatic invasion, vascular invasion, neural invasion, margins status, adjuvant therapy and survival (DFS-disease free survival). For 23 patients the clinical follow-up was not available in the archives of CHUV and we have contacted their physician. The most

parameters have been analyzed as numerical values. Due to limited number of patients, and in order to perform pertinent statistical analysis, several data were regrouped. Such as, the high-grade tumors (G2 and G3) were grouped and compared to low grade tumors (G1). The tumor stages T1/2 were also grouped and compared to and T3/4 stages. The IPMN lesions were classified as low-grade (adenoma) and high grade (borderline neoplasia and carcinoma in situ). The margins status were classified as margins >0,1cm and margins ≤0,1cm.
Clinical follow-up has been analysed according to tumor-free survival, survival with disease, death without disease and death with disease.

4.Result

The follow-up in our study ranges from 1 month to 7 years, with a mean follow-up of 20 months.
A univariate statistical analysis was conducted in order to compare the influence of each of the above mentioned variables in order to determine their impact on patient survival.
After that, a multivariate analysis was performed in order to determine which of all parameters confounded had affected significantly the patient survival in each group (adenocarcinomas on PanIN and adenocarcinomas on IPMN).
Table 2 shows distribution of all tumoral parameters analysed in our study

4.2 Table 2: Distribution of all tumoral parameters

	Adenocarcinomas on PanIN (n=22)	Adenocarcinomas on IPMN (n=13)
Gender :		
Men (n=20)	12	8
Women (n=15)	10	5
Age :		
<70 (n=18)	11	7
>70 (n=17)	11	6
PanIN1-A and B (n=25)	16	9
PanIN 2+3 (n=33)	23	10
IPMN Adenoma (n=9)	0	9
IPMN Borderline+CIS (n=9)	0	9
T1/2 stage (n=8)	3	5
T3/4 stage (n=25)	19	6
Lymph node metastases		
N+ (n=20)	16	4
N- (n=15)	6	9
Metastases		
M+ (n=5)	3	2
M- (n=30)	19	11
Tumor grade		
G1 (n=7)	1	6
G2/G3 (n=28)	21	7
Lymphatic invasion		
Yes (n=21)	17	4
No (n=14)	5	9
Vascular invasion		
Yes (n=14)	10	4
No (n=21)	12	9
Perineural invasion		
Yes (n=28)	20	8
No (n=7)	2	5
Margins		
$\leq$0,1cm (n=18)	13	5
>0,1cm (n=17)	9	8
Adjuvant therapy		
Yes (n=19)	14	5
No (n=16)	8	8

From 35 patients involved in this study, 22 presented ductal adenocarcinomas on PanIN (Figure 1a) and 13 adenocarcinomas on IPMN (including 6 ductal adenocarcinomas, 5 mucinous adenocarcinomas and 2 adenomas). (Figures 1b,1c).

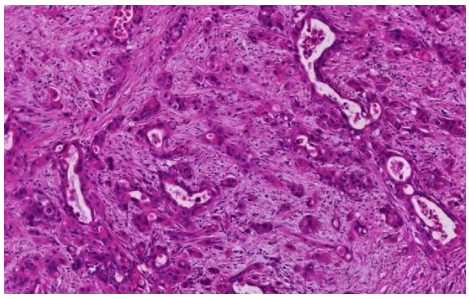


Figure 1a : Ductal adenocarcinoma on PanIN (original magnification 7x400)

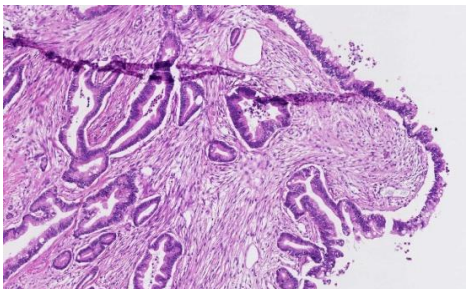


Figure 1b : Ductal adenocarcinoma on IPMN (original magnification 8x400)

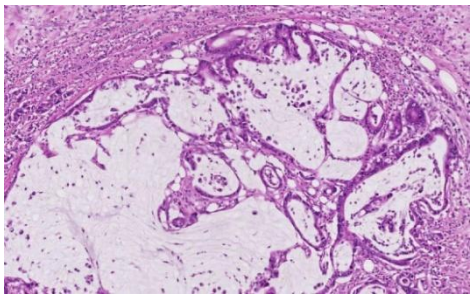
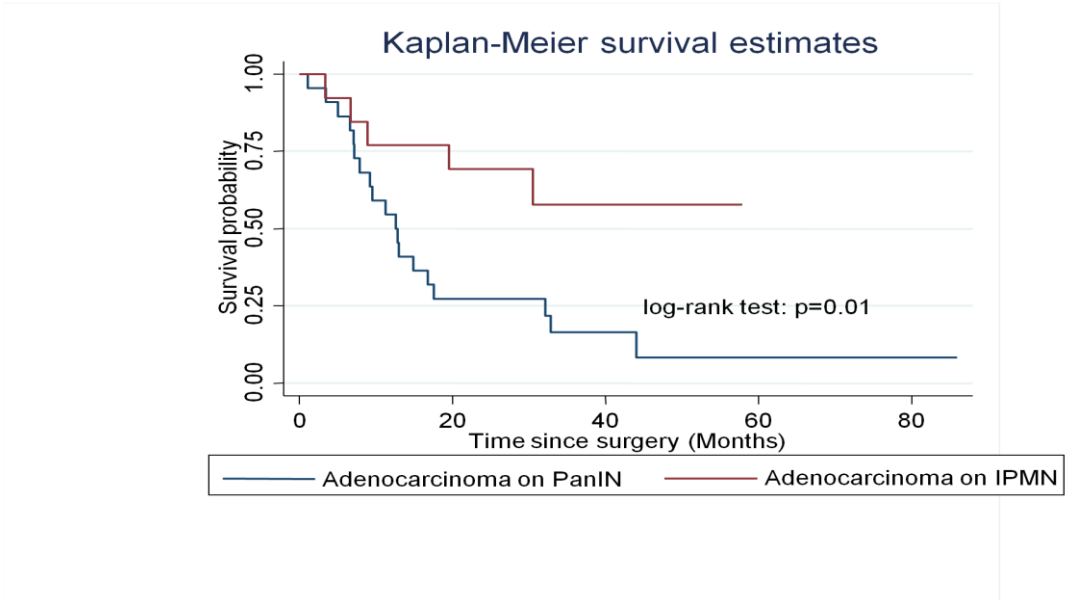


Figure 1c : Mucinous adenocarcinom on IPMN (original magnification 7x400)

The patient's mean age was 69 years, and the mean survival was 20 months. The most of patients (22) presented ductal adenocarcinomas developed on PanIN with a more aggressive behaviour, which can explains in part a mean survival at about only 20 months.

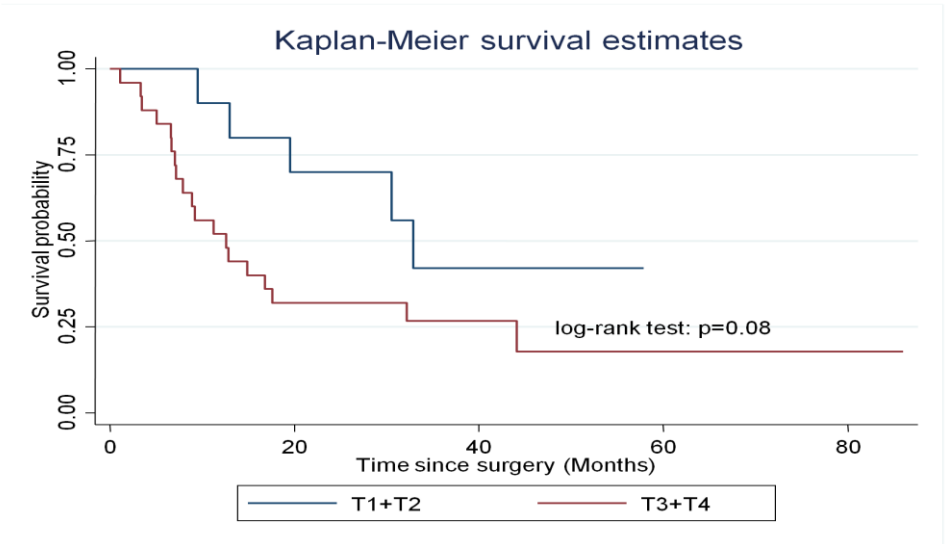
The comparative analysis of survival between adenocarcinomas on PanIN and adenocarcinomas on IPMN is shown in **Figure1**. The patients with an adenocarcinoma on IPMN present significantly better overall survival than those presented an adenocarcinoma developed on PanIN (**P value =0,01**).

Figure 1



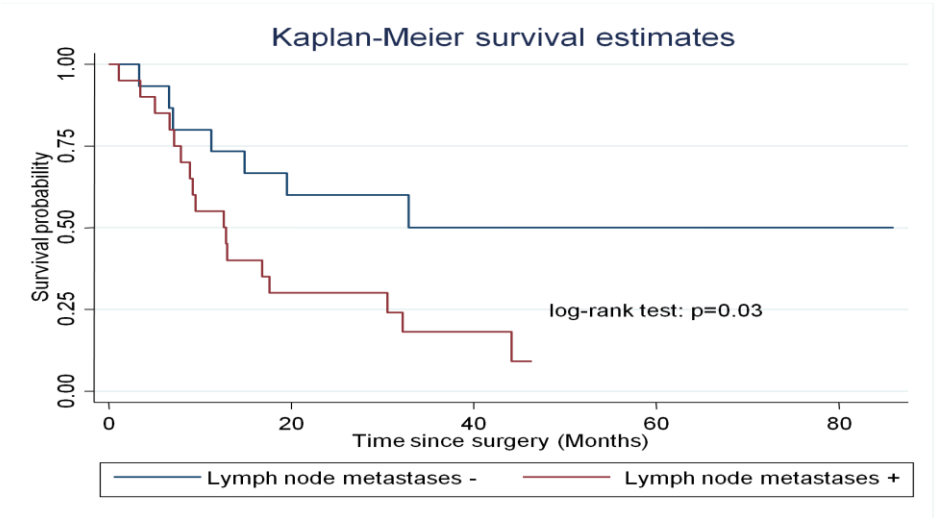
8 patients presented T1/2 stage and 25 patients presented a T3/4 stage. The survival for T1/2 group is improved as compare to T3/4 (**P value=0,08**) as shown in **Figure 2**.

Figure 2



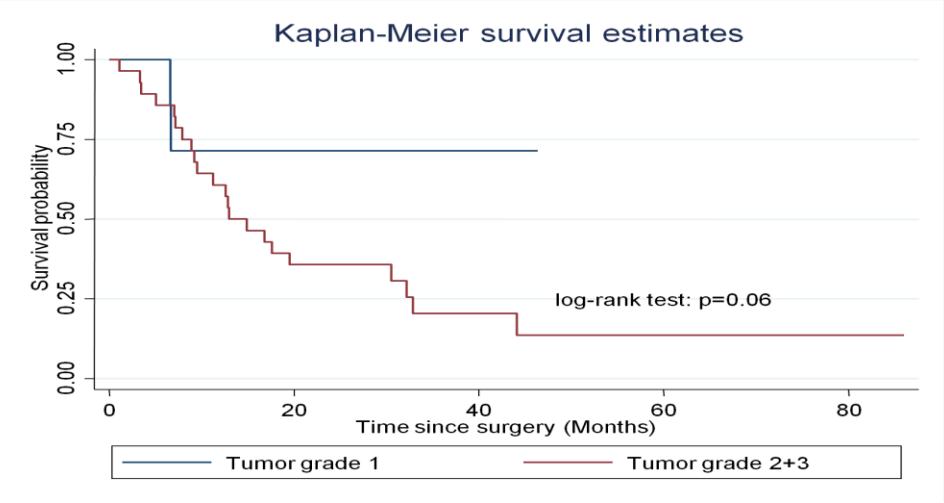
20 patients presented lymph node metastases. The survival for patients without lymph node metastases is improved as compared to patients with lymph node metastases (**P value = 0,03**) as shown in **Figure 3** .

Figure 3



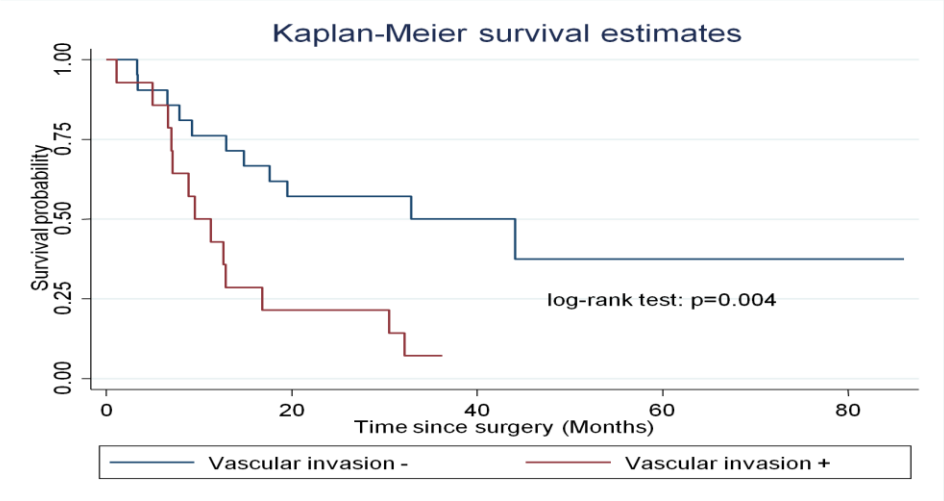
The tumor grade is analyzed by comparing G1 against G2/3 as shown in **Figure 4**. 7 patients presented G1 grade, while 28 patients presented G2/3 grade. As expected, the overall survival is higher in low-grade tumor group (G1) as compared to high-grade group (G2/3) (**P value= 0,06**) .

Figure 4



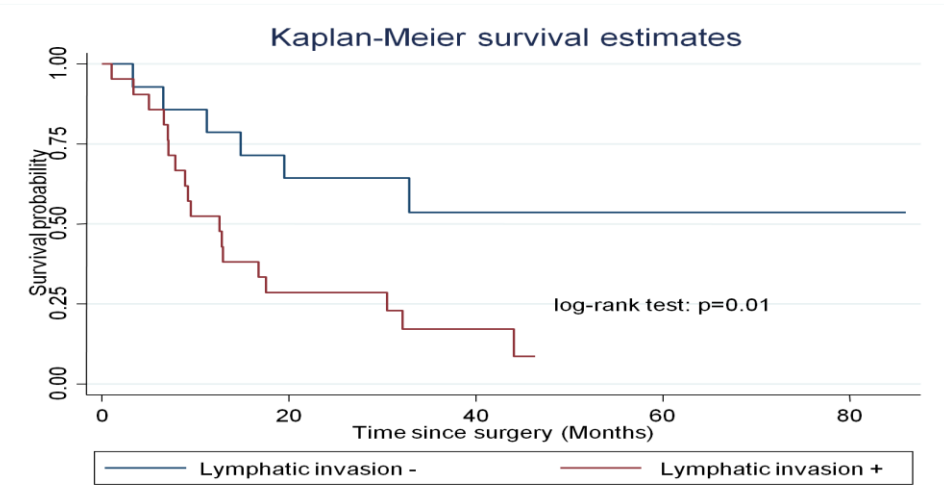
14 patients presented adenocarcinoma with vascular invasion. The survival for patients without vascular invasion is improved as compared to patients with vascular invasion (**P value=0,04**) as shown in **Figure 5**.

Figure 5



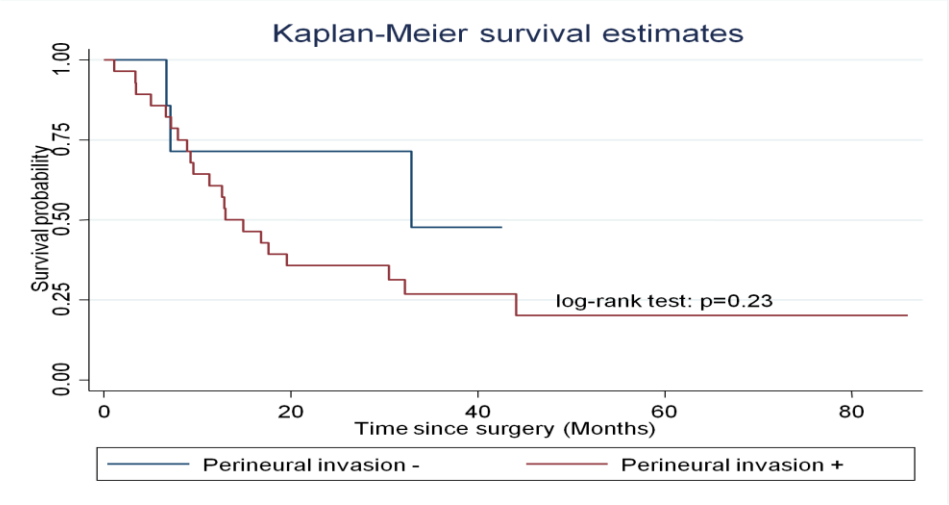
21 patients presented adenocarcinoma with lymphatic invasions. The survival for patients without lymphatic invasion is improved as compared to patients with lymphatic invasion (**P value=0,01**) as shown in **Figure 6**.

Figure 6



28 patients presented adenocarcinoma with perineural invasion. The survival for patients without perineural invasion is improved as compared to patients with perineural invasion (**P value = 0,23**) as shown in **Figure 7**.

Figure 7



18 patients presented resection margins ≤0,1/cm. The survival for patients with margins >0,1cm is improved as compared to patients with margins ≤0,1cm/(**P value=0,752**, data not shown)

9 of 35 patients beneficed the adjuvant chemotherapy. In our collective, the survival was improved for patients without chemotherapy as compared to patients with chemotherapy. (**P value =0,959**, data not shown).

The distant metastases analysis is not presented here, as the number of patients with distant metastases was very limited (only 5 of 35) to conduct a pertinent statistical analysis.

The multivariate analysis revealed one parameter influencing significantly the survival. It appears that vascular invasion, compared to all other factors, is associated to poor survival (**P value=0,03**).

14 of 35 patients involved in our study presented vascular invasion. Between them, 10 patients (45%) presented adenocarcinoma on PanIN and only 4 patients (30%) presented adenocarcinoma on IPMN. As the vascular invasion will promote the development of distant disease, this distribution should explain in part a mean survival at about 20 months in our collective.

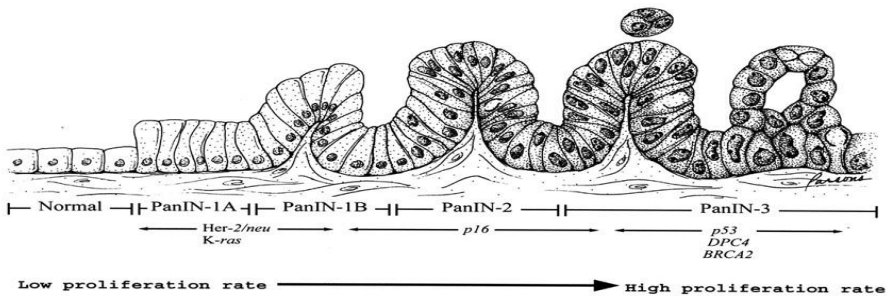
5. Discussion

Initially we aimed to perform a comparative analysis between ductal and mucinous subtype of pancreatic adenocarcinomas. However, the number of patients with purely mucinous adenocarcinomas was limited to conduct a statistical analysis. That's why we decide to perform a comparative analysis between adenocarcinomas developed on PanIN and adenocarcinomas developed on IPMN.

The both type of pancreatic adenocarcinomas affect an elderly population, with a mean age of 69 years. The poor prognosis is most often observed in group of adenocarcinomas developed on pre-existing PanIN lesions. The average survival was approximately 27% at 20 months.

Our study reflects that adenocarcinomas (ductal or mucinous) developed on IPMN are associated with better survival and prognosis than ductal adenocarcinomas arising in PanIN lesions.
This fact should be in part explains by different molecular events and alterations during tumour progression.
The adenocarcinoma of ductal type develops along the adenoma-carcinoma sequence. During this transformation several genetic alterations occur. The earliest and most frequent genetic alterations in PanIN lesions are the inactivation of K-RAS and telomerase shortening, while the inactivation of p53, SMAD4 and BRCA2 occurs more often with progression into PanIN2 and PanIN3 lesions (1)(2). The SMAD4 inactivation, usually found in PanIN, is rare in IPMN. The major molecular alterations are shown in **Figure 8**.

Figure 8: Correlation between proliferative activity and dysplasia in pancreatic intraepithelial neoplasia (PanIN). *Ref: Walter M Klein, Ralph H Hruban, Andres J P Klein-Szanto and Robb E Wilentz*



Progression Model of Pancreatic Cancer

Actually, the sequence of genetic alterations for IPMN is less well known. 1/3 of IPMN present an alteration of the gene STK11/LKB1, while this it is unusual in the PanIN (1).
We can therefore make the assumption that these different genetic alterations may influence the evolution in ductal or mucinous types adenocarcinomas and the degree of tumor aggressiveness.

Another explanation of difference in suvival and behaviour between these two histological entities is related to their morphological characteristics.
Adenocarcinomas developed on IPMN are usually associated with cystic structures easily visible on imaging. Therefore these lesions should be potentially detected and treated at an earlier stage than conventional ductal adenocarcinomas developed on PanIN lesions. This fact should also explain a difference in survival between adenocarcinomas on PanIN and adenocarcinomas on IPMN. This fact is also supported in our study. The analysis of TNM stage shows that only 3 (13%) adenocarcinomas developed on PanIN are at T1/2 stage, while in group of adenocarcinomas developed on IPMN they represent 5 (38%) of adenocarcinomas.
The closely similar results were found after analysis of lymph node metastases. 9 (69%) of adenocarcinomas developed on IPMN were free of lymph node metastases, while only 6 (27%) of adenocarcinomas developed on PanIN do not present metastatic lymph node disease.

In our study 19 of 35 patients have been treated with adjuvant chemotherapy. It represents 14 (63%) of adenocarcinomas developed on PanIN, and only 5 (38%) of adenocarcinomas developped on IPMN. Usually it is rather for advanced cancers, such as adenocarcinomas on PanIN, that an adjuvant chemotherapy is applied. As the most of the patients with adenocarcinomas developed on IPMN were diagnosed at an earlier stage they were not treated with chemotherapy.

Concerning the distant metastatic disease, our study is not completely in agreement with data reported in literature (1)(5). In the most of cases pancreatic adenocarcinomas are diagnosed with metastatic disease at initial presentation. In our study only 5 (14%) of 35 patients presented distant metastases. However this parameter is not considered in our statistical analysis due to the limited number of patients.

This study shows a difference in survival between adenocarcinomas developed on PanIN and adenocarcinomas developed on IPMN (including both, ductal and mucinous type). It is very

interesting to note that both, mucinous and ductal adenocarcinomas developed on IPMN, are associated with a better survival as compared to ductal adenocarcinomas developed on PanIN.

Indeed, the IPMN lesions are different from PanIN lesions, due to their topography, morphology but also at the molecular level. These differences may in part explain the differences in behaviour and improved survival in patients with adenocarcinomas developed on IPMN. It is known that different IPMN subtypes will evolves toward mucinous or ductal adenocarcinoma, but the pathways is not still well known.

It will be interesting to study and compare the differences at the molecular level, firstly between ductal and mucinous adenocarcinomas developed on IPMN and secondly between ductal adenocarcinomas developed on IPMN and ductal adenocarcinomas developed on PanIN in order to predict tumour progression and prognosis.

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